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DIVERSITY OF ENDOPHYTIC FUNGI IN *PONGAMIA PINNATA* OF ACHANAKMAR TIGER RESERVE OF CHHATTISGARH

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ABSTRACT

Biodiversity of endophytic fungi of different root, leaf and bark samples viz. young, mature, dry, and infected of *Pongamia pinnata* was carried out in the Botany laboratory, Dr. C.V.R.U. Kargi Road Kota Bilaspur. Agar plate method and moist chamber techniques were used to isolate the endophytic fungi. During the study period, a total of 11 fungi were isolated under 10 genera from both agar plate and moist chamber method of which, 10 species of 8 genera were recorded from agar plate and 9 species of 8 genera were recorded from moist chamber. It was found that, *Alternaria alternata* was recorded in all the leaf samples and some bark samples to infect of the medicinal plant *Pongamia pinnata*. In agar plate method, *Alternaria alternata*, white sterile mycelia, *Penicillium funiculosum*, *Cladosporium cladosporiodes*, *Curvularia lunata*, green sterile mycelia were recorded from all the leaf samples. In moist chamber technique, *Colletotrichum falcatum* sterile mycelia, *Alternaria alternata* and some other fungi were recorded in all the leaf and some bark samples. It showed that infected leaves of the plant harbored maximum number of endophytic fungi followed by mature and young leaves. *Alternaria alternata* and white sterile mycelia were predominant in the agar plate method.

KEYWORDS: Endophytic fungi, medicinal and economic plant Pongamia pinnata, Agar plate method.

INTRODUCTION

The term “endophyte” originally introduced by de Bary (1866) refers to any organisms occurring within plant tissue, distinct from the epiphytes that live on plant surfaces. Endophytes have been defined by various scientists as mutualists that colonize aerial parts of living plant tissue and do not cause symptoms of disease. Mycorrhizal are endophytes but

from a special type which produces external structures from the host plants (Mishra *et al*. 2014). Endophytic fungi are a group of fungi that colonize living and internal tissue of plants without causing any immediate, overt negative effects. Endophytic fungi represent an important and quantifiable component of fungal biodiversity, and are known to affect plant community diversity and structure. (Padhi et al. 2013).

Fungi are ubiquitous and morphologically diverse in nature, they occur in wide spectacular array of shapes, size, and colors. They have unique physiological and biochemical properties. Studies on endophytic fungi are over the past twenty years indicate that they occupy a unique ecological niche and are thought to influence plant distribution, Ecology, Physiology and Biochemical. (Nayak 2015).Endophytes, microorganisms that reside in the tissues of living plants are relatively unstudied and potential sources of novel natural products for exploitations in medicine, agriculture and industry (Schulz *et al.* 2002). *Pongamia pinnata* L. commonly known as Karanja (Family - Leguminaceae) is one of the important plant of high commercial value. It has been recognized as a major biodiesel producer in India (Tiwari and Chittora, 2013).

MATERIALS AND METHODS

Study site: Study site the Achanakmar Wildlife Sanctuary is an Indian sanctuary in Mungeli district, Chhattisgarh state. It had been established in 1975, under the provisions of the Indian Wildlife Protection Act of 1972, and declared a Tiger Reserve under Project Tiger, in 2009. It is a part of the Achanakmar-Amarkantak Biosphere Reserve.

The typical monsoon climate of the reserve has three distinct seasons: Summer (March-June), Rainy (July-October), and winter (November-February). The months of May and June are generally the hottest in the reserve, while the coolest months are December and January. The south-western monsoon brings rainfall to the area during the months of June to September.

Collection of plant samples: Plant samples (*Pongamia pinnata*) from unique environment niches of Amarkantak Tiger Reserve region. Mature and healthy plant leaves, stems and roots of *Pongamia pinnata* were collected by sampling different parts of the trees of forest area, Amarkantak Achanakmar. Especially those with an unusual biology and possessing novel strategies for survival were selected for study department of botany in aseptic condition the completion of the experiment. The samples were collected in sterilized polythene bags from

the different location of Achanakmar region during the months of August –November. (Arya et al. 2015).

Isolation of endophytic fungi: Plant material was thoroughly washed with running tap water, cut under sterile condition into small pieces of (2-3 cm) and surface sterilization with 1% sodium hypochlorite followed by 75% ethanol. Trace of both the treatment viz. sodium hypochlorite and alcohol were removed with a rinse in sterile distilled water. The plant segments were then plated on different microbial media such as water agar, potato dextrose agar and raised Bengal agar. (Tiwari et al.2013).

The plates were incubated at 25 c. for one week. Hyphal tips of fungi, emerging out of the plant tissue, were picked and grown on potato dextrose agar in pure culture. Resulting fungal colonies were purified and identified using their microscopic characters such as conidiophores and conidial structure measurement etc. Slants were prepared and stored at 4 c. until use. After the proper incubation of the plates, are set of seven days old cultures on PDA slant were preserved in mineral oil.

RESULT AND DISCUSSION

Different endophytic fungi were made from medicinal plant *Pongamia pinnata* from Isolation, Culture, and Identification techniques. 11 Endophytic fungal species were isolated under 10 genera from both Agar plate method and 9 species of 8 genera were recorded from moist chamber technique. From the leaf samples two *Trichoderma* sp. viz. *Trichoderma harzianum* and *Trichoderma* sp. were isolated. In table 1 given endophytic fungi isolated from *Pongamia pinnata* in Agar plate technique. In table 3 showed the incidence of endophytic fungi from moist chamber technique. It was found that, *Alternaria* sp. was recorded in all the leaf samples and *Curvularia lunata* was recorded in root and some bark samples to infect of the medicinal plant *Pongamia pinnata.* In Agar plate technique *Alternaria* sp., *Curvularia* sp., *Cladosporium* sp., *Penicillium* sp., white sterile mycelia, were recorded in all the leaf samples. In moist chamber, *Alternaria* sp., *Cladosporium* sp., *Colletotrichum* sp., green sterile mycelia were recorded in all the leaf samples and some root samples. Starting from young to infected of the plant *Pongamia pinnata*.

Table. 1: Incidence of endophytic fungi isolated from different leaf samples of *Pongamia pinnata* by agar plate technique.

Sl. No.	Endophytic fungi	Leaf samples		
		Young/mature	Dry	Infected
1.	Alternaria sp.	+	+	+
2.	Curvularia sp.	+	+	+
3.	Curvularia sp.	_	+	_
4.	white sterile mycelium	_	_	+
5.	Aspergillus sp.	+	_	+
6.	Cladosporioides sp.	+	_	_
7.	Aureobasidium sp.	+	_	+
8.	Curvularia sp.	_	+	_
9.	Trichoderma sp.	+	+	+
10.	Trichoderma sp.	+	+	+
11.	Nigrospora sp.	_	_	+

Table. 2: Incidence of endophytic fungi isolated from different root and bark samples of *Pongamia pinnata* by agar plate technique.

Sl. No.	Endophytic fungi	Root and Bark samples	
		Young	Infected
1.	*Trichoderma* sp.	+	+
2.	*Trichoderma aureoviride*	+	+
3.	*Colletotrichum* sp.	_	+
4.	*Cladosporiodes* sp.	_	+
5.	*Curvularia* sp.	_	+

Table. 3: Incidence of endophytic fungi isolated from different leaf, root and bark samples of *Pongamia pinnata* by moist chamber technique.

Sl. No.	Endophytic fungi	Leaf samples			Root and bark	
		Young/ mature	Dry	Infected	Young	Infected
1.	Alternaria sp.	+	_	+	+	+
2.	Colletotrichum sp.	_	_	+	_	+
3.	Cladosporiodes	_	_	+	_	+
4.	Green sterile mycelia	+	_	+	+	_
5.	Curvularia lunata	_	+	+	+	_
6.	Trichoderma sp.	_	_	+	+	+
7.	Pink sterile mycelium	+	_	_	_	+
8.	Curvularia catenulata	_	+	_	_	+
9.	Helminthosporium sp.	_	_	_	+	+

DISCUSSION

The leaf lamina of mature leaves in Sal, Harra and Bahera and its dorsi-ventral position provides a large surface area for the infection of endophytes. Furthermore, the above tree

species are semi evergreen in which leaves persist for several months (Sharma. 2015).The result of the present that diversity of endophytic fungi isolated from leaf, root and bark restricted the growth plant P*ongamia pinnata* (Nayak et al 2016). The natural products derived from microorganism and plants have been useful for treatment of various diseases and a large number of modern drugs have been developed. Diverse endophytic fungi reside in plants representing a rich resource of bio-active natural products with potential for exploitation in pharmaceutical & agricultural arenas (Anand D. et al 2014).

Endophytic and ectophytic organisms have received considerable attention as they are found to protect their host against pest pathogens and even domestic herbivorous. Fungi have been widely known as a source of bioactive compounds, an excellent example for the anti-cancer drug taxol, which was previously supported to occur only in the plant. The aim of this study was to establish any patterns in the distribution of endophytic fungal species from different leaf samples of *Pongamia pinnata* as well as the succession of endophytic fungi adhered to the leaves based on the ageing of the plant. (Paul et al. 2017).

CONCLUSION

A complete study on the endophytes of higher plant species with special reference to *Pongamia pinnata* plant has not been studied. The rapid research should be required to study them because disappearance of plant species will also disappears the entire suit of associated potential endophytes. By collecting, cataloguing and exploiting endophytic microorganisms throughout the world may offer opportunities in the field of industry and medicine.

Endophytic fungi are a rich and reliable source of novel natural compounds with interesting biological activities, a high level of biodiversity and may also produce several compounds of pharmaceutical significance, which is currently attracting worldwide scientific investigation toward isolation and exploration of their biotechnological promise. The future work pertaining to isolation endophytic fungi from plant parts may kindly be carried out by moist chamber methods than agar plate method (Padhi et al., 2013).

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